Epidemiology of *Staphylococcus aureus* bacteraemia at a tertiary children's hospital in Cape Town, South Africa

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DECLARATION

I, Reené Naidoo, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university, or been published prior to registration for the abovementioned degree.

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ABSTRACT

Background: *Staphylococcus aureus* is an important pathogen in paediatric patients with bloodstream infections. The epidemiology of *S. aureus* bacteraemia (SAB), however, has not been well documented in children in South Africa.

Methods: A retrospective study was conducted at a children's hospital in Cape Town, South Africa, to investigate the epidemiology of SAB from 2007-2011. The incidence, clinical presentation, risk factors, management and outcomes of methicillin sensitive *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) bacteraemia were compared.

Results: Over the five year study period, 365 episodes of SAB were identified. The annual incidence of SAB was 3.28 cases per 1000 hospital admissions. MRSA was responsible for 26% of SAB and 72% of nosocomial infections. Only six possible cases of community-acquired MRSA infections were described. MSSA bacteraemia was more likely to present as pulmonary and bone or joint infections, while bacteraemia without a source was the most common presentation with MRSA. Infants, children with malnutrition, and residents of long-term care facilities were at highest risk for MRSA bacteraemia. The overall case fatality rate for SAB was 8.8% over five years, with MRSA being the only significant risk factor for mortality.

Conclusion: The incidence of SAB and MRSA bacteraemia in children has remained stable over the past five years. MRSA is a predominantly nosocomial pathogen in children with SAB in Cape Town, South Africa.

PART A: STUDY PROTOCOL

Epidemiology of *Staphylococcus aureus* bacteraemia at a tertiary children's hospital in Cape Town, South Africa

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INTRODUCTION

Staphylococcus aureus is a ubiquitous gram-positive bacterium known to colonize the skin and mucous membranes of healthy individuals, with primary sites being the anterior nares, the axilla, perineum and rectum. It is also a major human pathogen capable of causing a wide variety of infections ranging from skin and soft tissue infections to life-threatening invasive disease. This highly adaptive organism has become a significant source of nosocomial and community-acquired infections worldwide.

Methicillin resistant *S. aureus* (MRSA) was first described in 1961, soon after the introduction of anti-staphylococcal penicillins (Zetola et al., 2005). Methicillin resistance is conferred by a penicillin-binding protein, PBP2A, encoded by the *mec*A gene located on the staphylococcal cassette chromosome (SCC) *mec*. PBP2A allows bacterial cell wall biosynthesis despite the presence of beta-lactam antibiotics (Bassetti et al., 2009).

Traditionally, MRSA has been associated with healthcare-associated infections. Specific risk factors for healthcare-associated infection include hospitalization or surgery within one year of infection, recent out-patient hospital visit, residence in a long-term care facility, recent antibiotic exposure, chronic illness or close contact with a person with any of these risk factors (Aires de Sousa & de Lencastre, 2004; Bassetti et al., 2009). Since the late 1990's, however, community-acquired MRSA infections have been rapidly emerging (Aires de Sousa & de Lencastre, 2004). Community-acquired MRSA (CA-MRSA) is distinguished from healthcare-associated MRSA (HA-MRSA) by the absence of any of the aforementioned risk factors, a distinct antimicrobial sensitivity pattern and a different genetic background. In contrast to HA-MRSA, which is generally multi-drug resistant, CA-MRSA has been noted to be susceptible to multiple non-beta-lactam antibiotics such as clindamycin, fluoroquinolones, gentamicin, trimethoprim-sulfamethoxazole, and rifampicin. Molecular genetic typing has also shown that HA-MRSA possesses unique SCC*mec* types I-III, whereas CA-MRSA carries SCC*mec* types IV and V and also the gene for the unique Panton-Valentine leukocidin exotoxin (Bassetti et al., 2009).

The incidence of infections caused by *S. aureus* and MRSA in particular, has been increasing rapidly throughout the world. A recent international multi-centre study reported rates of MRSA in South Africa as high as 39% (Zinn et al., 2004). In addition, the Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) study, conducted in 17 different countries, showed a MRSA rate of 33% in South Africa, which was comparable to the mean worldwide incidence of 32% (Bouchillon et al., 2004). Local studies conducted in Johannesburg and KwaZulu-Natal, showed rates of MRSA of 23% and 27% respectively (Perovic et al., 2006; Shittu & Lin, 2006).

The antimicrobial resistance patterns of *S. aureus* differ significantly based on whether the strain is healthcare-associated or community-acquired. A recent study investigating the antimicrobial sensitivity of MRSA isolates throughout South Africa showed high rates of resistance to non-beta-lactam antibiotics, with most isolates being multi-drug resistant (Marais et al., 2009). Notably, differing antibiotic profiles were recorded between the public and private health care systems. Significantly higher rates of antibiotic resistance to rifampicin, gentamicin and trimethoprim-sulfamethoxazole were noted in the public sector, while higher rates of resistance to ciprofloxacin, clindamycin and erythromycin were noted in the private health sector. These different resistance patterns were attributed to variation in disease profiles and empirical antibiotic choices amongst clinicians in the public and private sectors.

The majority of studies on *S. aureus* in South Africa were conducted on both adults and children. Few local studies have characterized *S. aureus* infections in children alone. A

recent study on community-acquired pneumonia at Red Cross Children's Hospital showed that *S. aureus* was one of the predominant pathogens isolated from upper and lower respiratory tract secretions in children with pneumonia, especially in those who were HIV positive (Zar et al., 2003). A similar study, conducted at Chris Hani Baragwanath Hospital in Johannesburg in children with lower respiratory tract infections, revealed that 60% of *S. aureus* isolates in HIV-infected children were MRSA (Madhi et al., 2000).

Clinical experience at Red Cross Children's Hospital suggests that the incidence of MRSA is low but there is no recent evidence to support this conclusion. Studies, showing MRSA rates of between 23% and 39% in large tertiary hospitals in South Africa, suggest that a detailed investigation of *S. aureus* infections in children at our institution is warranted.

AIM

To investigate the epidemiology of *S. aureus* bacteraemia in children at Red Cross Children's Hospital.

OBJECTIVES

1. To determine the rate of *S. aureus* bacteraemia per 1000 hospital admissions per year at Red Cross Children's Hospital over a 5 year period from January 2007 to December 2011.
    a. To determine the frequency of MRSA bacteraemia per year over the 5 year period.
    b. To determine the frequency of MSSA bacteraemia per year over the 5 year period
2. To compare the clinical presentations of MSSA and MRSA bacteraemia ,
3. To investigate the antibiotic sensitivity profiles of *S. aureus* bacteraemia isolates , and to describe current management at Red Cross Children's Hospital with regard to antibiotic choice, time to effective antibiotic therapy and duration of treatment,
4. To characterize the temperature patterns of children with *S. aureus* bacteraemia
5. To compare the clinical outcomes of patients with invasive MSSA and MRSA infections.

METHODS

Setting

The study will be undertaken at Red Cross Children's Hospital in Cape Town, South Africa. This is a tertiary level children's hospital serving all children up to the age of 13 years. It serves as a major referral centre in the Western Cape province of South Africa.

Study design

The study will be a retrospective review of all paediatric patients seen at Red Cross Children's Hospital with culture-confirmed *S. aureus* bacteraemia between January 2007 and December 2011. The National Health Laboratory Service (NHLS) microbiology database will be used to identify *S. aureus* blood culture isolates over the study period. Patient clinical records will be retrospectively examined and data collected on demographic information, diagnosis, onset of infection, site of infection, underlying illnesses, potential risk factors for HA-MRSA, antibiotic susceptibility profiles and clinical outcomes.

Definitions

1. *S. aureus* bacteraemia is defined as the isolation of *S. aureus* from a blood culture specimen.
2. The isolation of *S. aureus* from a blood culture drawn more than 48 hours after admission to hospital, or drawn at readmission within 48 hours of discharge, was considered to be a nosocomial infection. Healthcare-associated infections were defined as those that occurred within 48 hours of admission to hospital, with at least one of the following healthcare-associated risk factors: hospitalization or surgery within one year of onset of infection, presence of an invasive device at the time of admission, resident of a long-term care facility, and history of MRSA colonization or infection. Community-acquired infections were those identified within 48 hours of

hospital admission, but with none of the aforementioned healthcare-associated risk factors (Klevens et al., 2007).

3. HIV infection is determined by HIV PCR testing in children younger than 18 months of age and by HIV ELISA testing in children over 18 months of age.
4. Malnutrition according to WHO growth standards is defined as a weight-for-height or height-for-age z-score between -2 to -3 SD below the mean NCHS/WHO reference values for moderate malnutrition, while severe malnutrition is defined as a weight-for-height or height-for-age z score below -3SD of the mean, or by the presence of nutritional oedema (WHO, 1999).

Statistical analysis

Data will be analysed with standard statistical procedures. Analysis of variance (ANOVA) and unpaired t-tests will be used to analyse parametric variables. Non-parametric variables will be assessed with Mann Whitney or Kruskal Wallis tests. Categorical variables will be assessed with chi-square tests.

Ethical considerations

This study will be undertaken as a retrospective chart review. All records will be treated confidentially by the investigator. The study will be submitted for approval to the Departmental Research Committee, Department of Child and Adolescent Health, University of Cape Town, and the Research Ethics Committee of the Faculty of Health

Sciences, University of Cape Town. The study will be done in accordance with the Declaration of Helsinki (SA DoH, 2006).

The study poses no risks or direct benefits to the study participants as the investigation is retrospective. The anticipated benefit of this investigation is to contribute to scientific knowledge of *S. aureus* infections in a local context and facilitate clinical patient management.

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PART B: LITERATURE REVIEW

INTRODUCTION

Bloodstream infections are an important cause of serious and potentially life-threatening disease in children. *Staphylococcus aureus* is a common gram positive organism responsible for community and hospital-acquired infections in both adults and children. Worldwide, the spectrum of this highly adaptive organism has been changing rapidly over time. Within 30 years of the discovery of penicillin in the 1920's, penicillin-resistant *S. aureus* became pandemic, and strikingly, within 3 years after the introduction of semi-synthetic penicillin, methicillin-resistant *S. aureus* (MRSA) was first reported in England in 1961 (DeLeo & Chambers, 2009; Zetola et al., 2005). Since then, MRSA has become a dominant cause of nosocomial infections in hospitals throughout the world. In the early 1990's, however, the first report of community-acquired MRSA infections arose and again, these new strains of *S. aureus* have been reported globally (Bassetti et al., 2009).

While *S. aureus* infections in children have been described in many developed countries, the epidemiology is not well defined in South Africa. In order to improve our understanding and clinical management of this infection, it is vital to define the burden of disease locally. This literature review was conducted to establish the relative contribution of *S. aureus* to bloodstream infections in children in South Africa and in Africa as a whole, and secondly to evaluate the epidemiology of *S. aureus* bacteraemia

(SAB) in paediatric patients with regard to incidence, clinical presentation, risk factors, antimicrobial susceptibility patterns, clinical management and disease outcome.

LITERATURE RESEARCH STRATEGY

This literature search was conducted between November 2009 and June 2012 utilizing PubMed, with search terms “bacteraemia” and “Africa” in order to contextualize the contribution of SAB to all causes of bacteraemia in children in African countries. A second search was then performed with PubMed using the search terms “*Staphylococcus aureus*” and “bacteraemia”. In both search strategies, the studies were limited to those conducted in children under the age of 18 years and included original research, meta-analysis and review articles. Case reports were excluded from analysis. Additional articles were accessed with citation tracking from original work. Certain studies containing both adult and paediatric data were included if they pertained to the epidemiology of *S. aureus* infections in South Africa.

SUMMARY AND INTERPRETATION OF LITERATURE

Frequency of S. aureus as a cause of bacteraemia in Africa

Few South African studies have investigated the relative contribution of *S. aureus* to bacteraemia in paediatric patients. Cotton et al. (1992) described SAB to account for 11.6% of community and hospital-acquired bacteraemia in 165 children at a single tertiary hospital in Cape Town, while Berkowitz (1984) described an SAB rate of 6%

amongst 315 nosocomial cases of bacteraemia. Most other studies in South Africa were conducted on HIV-infected children. In 3 separate studies, SAB was reported to be responsible for 10.6%, 11% and 15.7% of bacteraemia in HIV-infected children (Cotton et al., 2008; Jaspan et al., 2008; Le Roux et al., 2011).

Bacteraemia in children has been more extensively investigated in other African countries compared to South Africa, although changes in the trends of bloodstream infections over time have not been well identified. Overall, a meta-analysis of community-acquired bloodstream infections identified SAB to be responsible for 12% of bacteraemia in children in Africa (Reddy et al., 2010). The frequency of SAB, however, varied greatly according to geographical region. In Malawi, only 1.9% of community-acquired bacteraemia were attributed to SAB (Walsh et al., 2000) while SAB rates of 7-9% were noted in Rwanda, Zimbabwe and Kenya (Lepage et al., 1987; Nathoo et al., 1996; Berkley et al., 2005; Brent et al., 2006). A higher SAB rate of 18.3% was described in Gambia and 20.9% in Nigeria (Hill et al., 2007; Obaro et al., 2011). Studies that have included both hospital and community-acquired bacteraemia, found an overall SAB rate of 12.5% in Kenya with 83% of these being community-acquired, 9% healthcare-associated and 8% nosocomial in origin (Aiken et al., 2011). Comparable findings were described in Tanzania, with SAB accounting for 10.2% of all bacteraemia, although in this study, 43% of SAB were community-acquired and 57% hospital-acquired (Blomberg et al., 2007). A single study conducted in Nigeria reported that SAB accounted for 48.7% of community and hospital-acquired bacteraemia (Meremikwu et

al., 2005). This high rate, however, was attributed to the high proportion of neonates included in this study.

Frequency of S. aureus bacteraemia

The epidemiology of SAB in children in South Africa has not been well described. A study conducted at Red Cross War Memorial Children's Hospital in 1974, reported on *S. aureus* infections in paediatric patients (Davis & White, 1974). This study included all *S. aureus* infections and was not specific to bacteraemia. At that time, 2% of isolates were reported to be MRSA. A second study, focusing on endocarditis in 36 children with SAB in Johannesburg, noted an MRSA rate of 31% (Friedland et al., 1995). No studies reported on the epidemiology of SAB and MRSA bacteraemia in South Africa from 1995 until 2004, when two international multi-centre studies reported rates of MRSA of 33.3%-39% in South Africa (Zinn et al., 2004; Bouchillion et al., 2004). This high rate of MRSA was further confirmed when a study from KwaZulu-Natal noted MRSA rates of 26.9% (Shittu & Lin, 2006). More recently, a national surveillance study on antimicrobial susceptibility revealed that 30-60% of *S. aureus* isolates in the South African public health sector were methicillin-resistant (Bamford et al., 2011). Most of these epidemiological studies included both adult and paediatric patients and do not provide an accurate quantification of MRSA in children in South Africa.

Studies in other African countries reported relatively low rates of MRSA in children. Three studies that investigated community-acquired SAB in Nigeria and Gambia

reported no MRSA isolates (Meremiku et al., 2005; Hill et al., 2007; Obaro et al., 2011). In Kenya, only 1% of SAB isolates were identified as MRSA, even though 10% of the SAB infections were nosocomial in origin (Ladhani et al., 2004). Only in Mozambique was there a report of a higher incidence of MRSA (8%) in children less than 15 years of age (Mandomando et al., 2010). A meta-analysis of community-acquired bacteraemia in adults and children in Africa found that 14% of SAB isolates were methicillin-resistant, but only 35 of 531 *S. aureus* isolates were tested for methicillin resistance in 22 different studies (Reddy et al., 2010). This systematic review appears to suggest that lack of detection rather than absence of disease may account for the low reported rate of MRSA bacteraemia.

Internationally, SAB has been extensively described. Three countries have conducted population based studies in children; in New Zealand the incidence of SAB was 16.9 per 100 000 population between 1996 and 1998 (Hill et al., 2001); in Denmark rates of SAB increased from 4.6 per 100 000 in 1971 to 8.4 per 100 000 population in 2000 (Frederiksen et al., 2007); in Calgary, Canada, SAB rates of 5.9 per 100 000 were reported between 2000 and 2006 (Vanderkooi et al., 2011). Most other investigations have reported the incidence of SAB within individual hospital settings; 3.7 cases per 1000 hospital admissions in California, USA (Burke et al., 2009), and 1.13 cases per 1000 paediatric admissions in Sydney, Australia (Suryati & Watson, 2002). MRSA rates as a proportion of SAB vary widely depending on geographical location. Denmark and Canada reported a very low frequency of 1% and 0.8% respectively (Frederiksen et al., 2007; Vanderkooi et al., 2011) compared to 6% in New Zealand and 14% in Australia

(Hill et al., 2001; Suryati & Watson, 2002). Data from the United Kingdom is variable. A surveillance study of MRSA bacteraemia in children noted an incidence rate of 1.1 per 100 000 child population per year between 2005 and 2007 (Johnson et al., 2010). Khairulddin et al. (2004) reported MRSA in England and Wales to have increased from 0.9% in 1990 to 13% in 2000, while Denniston & Riordan (2006) reported stable rates of MRSA of 5.8% in children and 27% in neonates at a single institution from 1993-2003. Adedeji and Gray (2005) also reported a stable rate of 15% between 1998 and 2003 at a single children's hospital. Rates of MRSA in the United States, however, have been increasing steadily over time. Wisplinghoff et al. (2003) reported MRSA bacteraemia to have increased from 10% in 1995 to 29% in 2001 in 49 paediatric hospitals throughout the country, while Burke et al. (2009) reported a similar increase of 9% in 2001 to 24% in 2006 in California.

Community-acquired MRSA (CA-MRSA) strains were first identified in Western Australia in 1993 and have been reported to be increasing throughout the world (Aires de Sousa & de Lencastre, 2004). CA-MRSA has been defined as MRSA infection acquired in the community or isolated within 48 hours after admission to hospital in patients without any healthcare-associated risk factors such as (1) previous hospitalization, dialysis or surgery within one year, (2) indwelling medical device at the time of admission, (3) resident of a long term care facility within one year or (4) history of MRSA colonization or infection (Klevens et al., 2007). CA-MRSA is postulated to have originated *de novo* in the community rather than spread from the hospital environment, as it has been described to have distinct genotypes and specific

antimicrobial susceptibility profiles (Shinefield & Ruff, 2009). Traditionally, the staphylococcal cassette chromosome (SCC) mec types IV and V, and strains producing the Panton-Valentine leukocidin toxin, have been associated with CA-MRSA isolates. However, recent evidence has shown that these molecular distinctions are becoming less clear (David et al., 2011). Most episodes of CA-MRSA are related to skin and soft tissue infections (Bassetti et al., 2009). CA-MRSA bacteraemia in children was reported to represent 4.7% of all invasive CA-MRSA infections in Taiwan (Chen et al., 2007), and 2.5%, 4.2% and 4.7% of all community-acquired *S. aureus* infections in children in Illinois, New York and Texas respectively (Sattler et al., 2002; Mongkolrattanothai et al., 2009; Suryadevara et al., 2010). Two South African studies have documented cases of CA-MRSA locally; one investigating MRSA isolates in both adults and children in Cape Town, identified 10 of 100 specimens to be CA-MRSA based on epidemiological criteria (Jansen van Rensburg et al., 2011) while an adult study on *S. aureus* bacteraemia reported 20% of their study population to have acquired MRSA in the community (Perovic et al., 2006). No other country in Africa has reported CA-MRSA in children.

Source of infection and clinical presentation

Both MSSA and MRSA bacteraemia are causes of nosocomial infection. In Australia, the United Kingdom and the United States, 47-52% of SAB infections were nosocomial in origin (Suryati & Watson, 2002; Denniston & Riordan, 2006; Burke et al., 2009). In contrast, only 10% of SAB in Kenya were hospital-acquired infections (Ladhani et al., 2004). This may be attributed to the high rates of co-morbid illnesses and hence hospital-exposure in children in the Australian, United Kingdom and United States-

based studies, compared to those in the Kenyan cohort. Hospital-acquired SAB occurred more commonly in children less than 1 year of age (Hill et al., 2001; Frederiksen et al., 2005). Suryati & Watson (2002) in Australia and Burke et al. (2009) in the United States found that MRSA contributed approximately 26% of nosocomial SAB.

The most frequent clinical diagnoses at the time of SAB were bacteraemia without focus, bone or joint infections, skin or soft tissue infections (SSTI) and respiratory tract infections. No focus of infection was detected in a third to a half of all SAB cases (Suryati & Watson, 2002; Ladhani et al., 2004; Denniston & Riordan, 2006). The incidence of endocarditis was low; 1.4% in Australia (Suryati & Watson, 2002), 1.7% in Denmark (Vanderkooi et al., 2011) and 1.3% in the USA (Burke et al., 2009). Notably, routine echocardiography was not performed in these studies. The findings of a paediatric study in South Africa, however, is cause for concern as it found clinically silent endocarditis in 11% of children hospitalized with SAB (Friedland et al., 1995). None of the children had clinical signs of endocarditis and only one had underlying congenital cardiac disease. Although the sample size was small with only 36 children being evaluated in the study, it does raise the question of whether echocardiography should be conducted routinely in children with SAB.

Only one study evaluated the response of infective markers and temperature to SAB infections (Suryati & Watson, 2002). Notably, 30% of patients with SAB did not mount a leucocytosis response to the infection, while 24% did not present with fever. This

absence of clinical and laboratory markers of infection would make the prediction of bacteraemia more difficult. Additionally, the presence of a focus of infection did not affect either temperature or white cell count responses.

Risk factors for SAB and MRSA

Malnutrition has been shown to be a significant risk factor for bacteraemia in children in South Africa, Kenya and Malawi (Cotton et al., 1992; Friedland, 1992; Norton et al., 2004; Berkley et al., 2005; Aiken et al., 2011). Only one study, however, evaluated malnutrition as a specific risk factor for SAB. Ladhani et al. (2004) reported SAB without focus, to occur more frequently in children with malnutrition in Kenya.

HIV infection has also been shown to be associated with bacteraemia in children in Africa (Nathoo et al., 1996; Berkley et al., 2005). The link between HIV infection and SAB specifically has not been described, although HIV-infected children were noted to be at higher risk for MRSA infections; 77%-100% of SAB were due to MRSA bacteraemia in HIV-infected children (Cotton et al., 2008; Jaspan et al., 2008; Le Roux et al., 2011). This is likely due to the higher risk of hospitalization in HIV-infected children and consequently an increased risk of hospital-acquired infections.

Generally, infants appear to have a higher incidence of SAB compared to older children (Hill et al., 2001; Suryati & Watson, 2002; Adedeji & Gray, 2005; Denniston & Riordan, 2006; Frederiksen et al., 2007; Burke et al., 2009). Infants may also be at higher risk of

MRSA bacteraemia, as described in England and Wales (Khairulddin et al., 2004). Several studies also described SAB as occurring more commonly in those with co-morbid conditions, such as congenital heart disease, malignancy and chronic skin disorders (Denniston & Riordan, 2006; Burke et al., 2009; Vanderkooi et al., 2011). With regard to MRSA infection, intravascular devices, most commonly central venous catheters, were the most commonly identified risk factor. Indwelling devices were responsible for 63% of MRSA infections in children and 88% in neonates in England (Adedeji & Gray, 2005; Denniston & Riordan, 2006). An Australian study, however, noted an equal incidence of MSSA and MRSA infections in children with central line infections due to SAB (Suryati & Watson, 2002).

Antimicrobial sensitivities

Several South African studies investigated antimicrobial susceptibility patterns for *S. aureus* in both adult and paediatric patients. With the exception of widespread resistance to penicillin (92%), most MSSA isolates were susceptible to the majority of antibiotics against which they were tested (Shittu & Lin, 2006). MRSA, however, displayed multi-drug resistance patterns with over 75% of isolates in KZN, and 55-78% of isolates nationally, being resistant to gentamicin, trimethoprim-sulfamethoxazole (TMP/SMX), erythromycin and clindamycin (Shittu & Lin, 2006; Marais et al., 2009).Susceptibility patterns of ciprofloxacin and rifampicin differed in the two studies; 69% versus 18% for ciprofloxacin and 38% versus 74% for rifampicin in the national and the KZN study, respectively. This may be attributed to the inclusion of both public and private sector MRSA isolates in the national study which demonstrated differing

antimicrobial susceptibility profiles between patients in these two sectors (Marais et al., 2009). Isolates from the private sector were more likely to be resistant to the commonly used antibiotics ciprofloxacin, clindamycin and erythromycin, while public sector patients were more likely to be infected with isolates that were resistant to gentamicin, rifampicin and TMP/SMX. In the public health service, gentamicin is a standard first line antimicrobial agent for the management of sepsis in children. Rifampicin is widely used as part of anti-tuberculosis therapy and TMP/SMX is a commonly used prophylactic agent against *Pneumocystis jiroveci* in HIV-infected patients. Unfortunately, clindamycin resistance rates were noted to be high in both the KZN and national studies, as well as in a more recent report by Bamford et al. (2011), who reported 55-88% clindamycin resistance across the country. This limits the usefulness of clindamycin against MRSA infections in the South African setting until susceptibility results are known. All *S. aureus* isolates tested were susceptible to vancomycin, teicoplanin and fusidic acid (Shittu & Lin, 2006; Marais et al., 2009). Based on these antimicrobial susceptibility patterns, *S. aureus* appears to be a predominantly hospital-acquired pathogen in South Africa. CA-MRSA isolates from other countries usually displayed increased susceptibility to non-beta-lactam antibiotics (Bassetti et al., 2009).

Management of SAB infections

Antibiotic choice and duration of treatment of SAB infections varied according to the clinical diagnosis at the time of bacteraemia. In Kenya, children with SAB without a focus were less likely to receive correct empiric antibiotics than those with a focus (Ladhani et al., 2004). Similarly, only 36% of patients in Australia and 69% of patients in

the United Kingdom received the correct empiric antibiotics, with 65% having received less than two weeks of effective therapy (Suryati & Watson, 2002; Denniston & Riordan, 2006). CA-MRSA infections were even less likely to have received the correct initial antibiotics – only 29% of children with CA-MRSA infections in Taiwan received successful empiric therapy (Chen et al., 2007).

In accordance with international standards of care, bone and joint infections were treated for the longest period - approximately 46-50 days (Hill et al., 2001; Denniston & Riordan, 2006). Bacteraemia without a focus, however, was only treated for 6 days on average in New Zealand (Hill et al., 2001) and 7.5 days in the United Kingdom (Denniston & Riordan, 2006). This is far shorter than the traditional 14 days of therapy for uncomplicated SAB. In addition, the Infectious Diseases Society of America recently published guidelines recommending that MRSA bacteraemia be treated for at least 14 days in the absence of any complicating factors (Liu et al., 2011).

Outcomes

SAB mortality rates differ between developed and developing countries. A population based study of SAB in children in Denmark over 30 years, showed that mortality rates dropped from 19.6% to 2.5% between 1971 and 2001 (Frederiksen et al., 2007). Case fatality rates between 0.7% and 3.2% were noted in New Zealand, Australia, England and the USA (Hill et al., 2001; Suryati & Watson, 2002; Denniston & Riordan, 2006; Burke et al., 2009). However, mortality was significantly higher in Kenya with a case

fatality rate of 24.7% (Ladhani et al., 2004). Children in the Kenyan study were at higher risk of death if there was no identified focus of SAB (46.7% mortality rate) compared to a 5.8% mortality rate in those with a focus of infection. Lack of appropriate empiric antibiotic therapy contributed to the higher risk of death in children without a focus of infection. Children with MRSA also had higher mortality rates - 7.3% in the United Kingdom, and 10% in the United States (Adedeji & Gray, 2005; Burke et al., 2009). Bacteraemia together with pulmonary infections were noted to contribute to mortality in infants and children over 10 years of age, while endocarditis was a significant risk factor for mortality in children less than 10 years (Frederiksen et al., 2007).

AREAS FOR FUTURE RESEARCH

This review has clearly demonstrated that *S. aureus* is a significant aetiological agent in paediatric bacteraemia in Africa, and the clinical epidemiology of this infection in children in South Africa warrants further investigation. Areas for future research should include the evaluation of the incidence of both community and hospital-acquired *S. aureus* bacteraemia in South African children. Reported rates of MRSA infection are high in combined adult and paediatric studies but there is a paucity of information on MRSA infection in children alone. Knowledge of local epidemiology, including spectrum of the disease, clinical presentation and local risk factors for SAB would influence empiric disease management of both community and hospital-acquired bacteraemia. CA-MRSA has been reported in only two South African studies. Additional detailed studies are required to identify the magnitude of this problem in children. Investigations

that combine clinical and molecular typing of *S. aureus* are essential to clearly describe CA-MRSA. Mortality in children with SAB is reported to be high in Africa and much lower in the developed world. With a burgeoning HIV epidemic and a high incidence of malnutrition, it is imperative to undertake studies that elucidate the relationship between these conditions and *S. aureus* bacteraemia and their combined effects on mortality.

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PART C: PUBLICATION-READY MANUSCRIPT

TITLE PAGE

Epidemiology of *Staphylococcus aureus* bacteraemia at a tertiary children's hospital in Cape Town, South Africa

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Key Words

Staphylococcus aureus; bacteraemia; children; MRSA

Abbreviated Title

Epidemiology of *Staphylococcus aureus* bacteraemia in children

Running Head Title

S. aureus bacteraemia in children

ABSTRACT

Background: *Staphylococcus aureus* is an important pathogen in paediatric patients with bloodstream infections. The epidemiology of *S. aureus* bacteraemia (SAB), however, has not been well documented in children in South Africa.

Methods: A retrospective study was conducted at a children's hospital in Cape Town, South Africa, to investigate the epidemiology of SAB from 2007-2011. The incidence, clinical presentation, risk factors, management and outcomes of methicillin sensitive *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) bacteraemia were compared.

Results: Over the five year study period, 365 episodes of SAB were identified. The annual incidence of SAB was 3.28 cases per 1000 hospital admissions. MRSA was responsible for 26% of SAB and 72% of nosocomial infections. Only six possible cases of community-acquired MRSA infections were described. MSSA bacteraemia was more likely to present as pulmonary and bone or joint infections, while bacteraemia without a source was the most common presentation with MRSA. Infants, children with malnutrition, and residents of long-term care facilities were at highest risk for MRSA bacteraemia. The overall case fatality rate for SAB was 8.8% over five years, with MRSA being the only significant risk factor for mortality.

Conclusion: The incidence of SAB and MRSA bacteraemia in children has remained stable over the past five years. MRSA is a predominantly nosocomial pathogen in children with SAB in Cape Town, South Africa.

INTRODUCTION

Staphylococcus aureus is a major human pathogen that causes a wide variety of infections ranging from skin and soft tissue infections to life-threatening invasive disease. This highly adaptive organism is a significant source of bacteraemia, responsible for both community-acquired and nosocomial infections. In Africa, several studies have investigated the incidence of both community and hospital-acquired bloodstream infections in children presenting with signs of systemic infection. The overall incidence of *S. aureus* bacteraemia (SAB) varies widely from 10% in Tanzania, to 12.5% in Kenya and 48.7% in Nigeria.[1-3] The high incidence noted in Nigeria however, is most likely due to the disproportionate number of neonates included in that study. Few South African studies have characterized the contribution of *S. aureus* to bacteraemia in children. In Cape Town, 11.6% of bacteraemia in hospitalized children were due to *S. aureus*, while SAB rates of 10.6%, 11% and 15.7% were reported in specific cohorts of HIV-infected children.[4-7]

Methicillin resistant *S. aureus* (MRSA) is one of the commonest aetiologic agents of healthcare-associated and nosocomial infection amongst both adults and children worldwide, and the incidence of MRSA has been increasing over the last few years.[8] Specific risk factors for healthcare-associated infection include hospitalization or surgery within one year of infection, residence in a long-term care facility, presence of an invasive device at the time of admission, and history of MRSA infection or colonization.[9] In the only two paediatric studies in South Africa on *S. aureus* infections, 2% of isolates

were reported as MRSA at Red Cross War Memorial Children's Hospital in 1974, while a second study, focusing on endocarditis in 36 children with SAB in Johannesburg, noted an MRSA rate of 31% in 1995.[10,11] More recently, an antimicrobial susceptibility study on blood culture isolates from children and adults in South African public hospitals in 2010, identified 30-60% of *S. aureus* to be MRSA.[12] Similarly, two international multi-centre studies reported MRSA to be responsible for 33% and 39% of *S. aureus* infections in South Africa.[13,14]

Since the late 1990's, community-acquired MRSA (CA-MRSA) infections have been reported to be rapidly emerging.[15] CA-MRSA is distinguished from healthcare-associated MRSA (HA-MRSA) by the absence of any of the aforementioned risk factors. CA-MRSA isolates are often associated with a distinct antimicrobial sensitivity pattern and a different genetic background, although this distinction is now becoming more blurred as cases of HA-MRSA caused by strains with genotypes similar to that described in CA-MRSA isolates have been described.[16] Reports of the prevalence of CA-MRSA vary widely across the world. Studies in the United States have reported CA-MRSA to account for 40% of MRSA infections in New England compared to 74% in Texas.[17,18] Notably, the majority of these infections (96-100%) were related to skin and soft tissue infections and not invasive disease. Bacteraemia due to CA-MRSA infections was reported to account for 4.7% of CA-MRSA in a Taiwanese study and 9.8% in the United Kingdom.[19,20] In South Africa, a study conducted in Cape Town identified 10 possible CA-MRSA amongst 100 MRSA isolates in both adults and children while an

adult study in Johannesburg reported 20% of MRSA bacteraemia to have originated from the community.[21,22]

The aims of this study were to investigate the epidemiology of SAB at a single children's hospital in South Africa over a five year period, and to describe the incidence, clinical presentation, microbiologic profiles, risk factors, management and outcomes of children with both methicillin-sensitive *S. aureus* (MSSA) and MRSA bacteraemia.

MATERIALS AND METHODS

Study population

This study was undertaken at Red Cross War Memorial Children's Hospital (RCWMCH), a tertiary level children's hospital in Cape Town, South Africa, which serves as a major referral centre in the Western Cape province for children up to 13 years. While the hospital does not provide routine postnatal care or have a dedicated neonatal intensive care facility, acutely ill neonates or those requiring paediatric surgery are managed at the hospital.

Study design

A retrospective analysis was conducted of all paediatric patients seen at RCWMCH with *S. aureus* bloodstream infections between January 2007 and December 2011. All patients from whom *S. aureus* had been isolated from blood culture specimens over the

five year period were identified using the National Health Laboratory Service (NHLS) database. Hospital clinical records were then retrospectively examined. Data were collected on demographics, clinical diagnosis, risk factors for infection, clinical and laboratory markers of infection, antibiotic susceptibility profiles, management of infection and clinical outcome.

Definitions

SAB was defined as the isolation of *S. aureus* from a blood culture specimen. Isolates were identified and tested for antimicrobial susceptibility at Groote Schuur Hospital NHLS microbiology laboratory, according to Clinical and Laboratory Standards Institute guidelines.[23] All *S. aureus* organisms cultured from blood specimens were considered to be clinically significant. However, multiple isolates of the same organism from the same patients were excluded from analysis if they were clinically deemed to be related to the incident infection. The isolation of *S. aureus* from a blood culture drawn more than 48 hours after admission to hospital, or drawn at readmission within 48 hours of discharge, was considered to be a nosocomial infection. Based on the definitions utilized by the Centers for Disease Control and Prevention, healthcare-associated infections were defined as those that occurred within 48 hours of admission to hospital, with at least one of the following healthcare-associated risk factors: hospitalization or surgery within one year of onset of infection, presence of an invasive device at the time of admission, resident of a long-term care facility, and history of MRSA colonization or infection. Community-acquired MRSA infections were those identified within 48 hours of hospital admission, but with none of the aforementioned healthcare-associated risk factors.[9]

Clinical diagnoses during the SAB episode were classified according to the assessment of the attending physician in the patient records. HIV infection was determined by HIV PCR testing in children younger than 18 months, and by HIV ELISA testing in children over 18 months. HIV exposure was identified by history of maternal HIV infection, or a positive HIV antibody test in infants less than 18 months who tested HIV PCR negative. Leucocytosis was regarded as a white cell count above the normal range for age according to the NHLS reference standards. Malnutrition was defined according to WHO growth standards as weight-for-height or height-for-age z-score -2 SD below the mean WHO reference values. Severe malnutrition was defined as weight-for-height or height-for-age z score below -3SD of the mean, or by the presence of nutritional oedema.[24]

Statistical analysis

Weight-for-age z-scores (WAZ), height-for-age z-scores (HAZ) and weight-for-height z-scores (WHZ) were calculated using Epi-Info, version 3.5.3 (2011). Data were analysed using Stata Statistical Software version 11.1 (StataCorp, College Station, Texas). Non-parametric variables were evaluated with Wilcoxon rank-sum tests and Kruskal-Wallis tests, and categorical data with chi-square tests. Odds ratios, together with 95% confidence intervals, were utilized to describe measures of effect. For all analyses, $p < 0.05$ was considered statistically significant. Logistic regression was performed to assess risk factors for MRSA infection and risk factors for mortality. Variables with $p < 0.1$ on univariate analysis were selected for stepwise forward multivariate analysis.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Departmental Research Committee of the Department of Child and Adolescent Health, and the Research Ethics Committee, Faculty of Health Sciences, University of Cape Town (HREC REF:029/2011).

RESULTS

Between January 2007 and December 2011, 365 episodes of *S. aureus* bacteraemia (SAB) were identified, 270 with MSSA and 95 with MRSA. Seventeen patients had two distinct episodes of SAB based on clinical presentation. *S. aureus* was isolated on subsequent culture specimens, including blood, bone, tissue, pus, pleural, pericardial, peritoneal and synovial fluids in 143/365 episodes (39%). Basic demographic (age, gender), laboratory and microbiological data were accessible for all SAB episodes. Unfortunately, due to the retrospective design of the study, clinical and outcome data were not available for all cases.

Incidence of infection

The annual incidence of SAB was 3.28 cases/1000 hospital admissions per year with a mean frequency of 2.43 MSSA cases and 0.85 MRSA cases per 1000 hospital admissions per year. Overall, MRSA was responsible for 26% of bacteraemia episodes due to *S. aureus*. There was no significant change in the frequency of SAB over the

study period (p=0.379) (Figure 1). While rates of MRSA infection remained fairly constant from 2007-2011, the incidence of nosocomial SAB rose steadily from 2007 and peaked in 2010 (Figure 2).

Characteristics of study population

Demographic characteristics and basic infective markers of the cohort are outlined in Table 1. The median age of staphylococcal bacteraemia was 11 months. Neonates accounted for 36/365 (9.9%) of episodes while infants represented 189/365 (51.8%) of SAB. Children with MRSA infection were significantly younger (p=0.026), and more frequently underweight (p=0.0000) and stunted (p=0.0000). In those children whose HIV exposure and testing status was known, 51 (19.8%) were HIV-infected and 48 (19.4%) were HIV-exposed but uninfected (Table 1). Notably 107 (29%) children had an unknown HIV status. HIV infection, but not HIV exposure, was significantly associated with MRSA bacteraemia (p=0.001). Analysis of infective markers, including white cell count (WCC), neutrophil count, band count and C-reactive protein (CRP), revealed no differences between MSSA and MRSA bacteraemia (Table 1).

Spectrum of S. aureus infections

The origin of SAB, was identifiable in 357/365 episodes: 33% (118/357) were nosocomial, 16% (56/357) healthcare-associated and 51% (183/357) community-acquired. MRSA was responsible for 21% of healthcare-associated and 72% of nosocomial infections. Six MRSA infections were classified as community-acquired with

no identifiable healthcare-associated risk factors (one in 2007, one in 2009, three in 2010 and one in 2011). Primary diagnoses for these cases were bacteraemia without identified source (4), gastroenteritis (1) and superficial skin sepsis (1).

Data on clinical diagnosis, available for 337 episodes of SAB (Table 2), showed that the most common clinical diagnosis was SAB without an identifiable source (33%), followed by SAB with pneumonia ± empyema (22%), SAB with skin and soft tissue infections (17%) and SAB with bone or joint infections (12%). Bloodstream infections without a source were more likely to be MRSA infections, while pulmonary, bone and joint infections were more likely to be due to MSSA. The only two bone or joint infections caused by MRSA were nosocomial in origin. Central venous catheter-related line sepsis was equally likely to be attributable to MSSA or MRSA, although all were nosocomial in origin (Table 2).

Risk factors for MRSA infection

Univariate analysis showed that prior hospitalization within one year, presence of an indwelling medical device, previous MRSA infection, being a resident of a long-term care facility, HIV infection, malnutrition, congenital cardiac disease, concurrent tuberculosis, duration of incident hospitalization and infancy (under 12 months), were significant risk factors for MRSA infection (Table 3). On multivariate analysis, infancy, resident of a long-term care facility, malnutrition, and longer duration of incident hospitalization remained significant risk factors.

Microbiologic profile

Results of antimicrobial susceptibility testing, available for all 365 isolates (Table 4), suggested that most MSSA isolates were susceptible to the majority of antibiotics tested. Only 6% of MSSA were susceptible to penicillin. Most MRSA isolates displayed multi-drug resistance. All MRSA were sensitive to vancomycin (minimal inhibitory concentration (MIC) ≤ 2µg/ml); three isolates were reported to have a vancomycin MIC equal to 2µg/ml. All three cases were treated successfully – catheter-related sepsis in a preterm infant with vancomycin alone, pneumonia in an HIV-infected child with vancomycin and clindamycin used sequentially, and bacteraemia without source in a child with short bowel syndrome with a combination of vancomycin and trimethoprim-sulfamethoxazole (TMP/SMX). Organisms isolated from HIV-infected patients were more likely to be resistant to TMP/SMX (63% vs. 21%) (p=0.000), gentamicin (55% vs. 24%) (p=0.000) and rifampicin (51% vs. 16%) (p=0.000). Of the 32 HIV-infected patients with TMP/SMX resistant isolates, 22 (69%) were on TMP/SMX prophylaxis at the time of SAB. Antimicrobial resistance patterns were similar in children who were HIV-exposed but uninfected, compared to children who were not exposed to HIV.

In 84/365 (23%) of SAB episodes, a concomitant organism was isolated on blood culture. Once typical skin contaminants (coagulase negative staphylococci, *bacillus spp.* and *corynebacterium spp*.) were excluded, 24/365 cultures (7%) had a clinically significant second organism (polymicrobial bacteraemia) – gram negative bacilli (10),

Enterococcus faecalis or faecium (9) and *Streptococcus* pyogenes *or agalactiae*. (5). Infants (11%), children with malnutrition (10.5%) and those with MRSA bacteraemia (12.6%) were more likely to have a significant concomitant organism on culture. No association was found between polymicrobial bacteraemia and HIV infection or HIV exposure on chi-square analysis.

Antibiotic management

Data on appropriate antibiotic therapy, which was regarded as the administration of antibiotics to which the organism was sensitive, were available for 322 episodes. At the time the culture was drawn, 31/322 (9.6%) were already on antibiotics to which the organism was sensitive and likely to respond clinically. The majority of these episodes (29/31; 94%) were caused by MSSA and 25/31 (81%) were community-acquired infections. Twelve of these patients received a dose of intramuscular ceftriaxone for suspicion of severe bacterial infection at a primary care clinic prior to transfer to RCWMCH, in keeping with South African Integrated Management of Childhood Illness guidelines.[25] The median time on therapy before the blood culture was drawn was 6 hours (IQR 1.5-22.7 hours).

Excluding those who were already on antibiotics at the time of culture, appropriate antibiotic therapy was instituted in 267/322 (82.9%) episodes of SAB. Time to appropriate therapy was calculated if the precise time of blood draw and time of antibiotic administration were documented. This information was available in 141

episodes. Appropriate therapy was initiated within a median time of 2.5 hours (IQR 0.7-8.5) in MSSA and 46 hours (IQR 4.7-55.3) in MRSA infections (p=0.0000). The median time to initiation of therapy was 2.5 hours for community-acquired infections, 5.8 hours for healthcare-associated infections, and 10.6 hours for nosocomial infections (p=0.0019). There was no significant difference in time to therapy when comparing episodes with an identified source of bacteraemia and those without.

The remaining 24/322 episodes (7.5%) did not receive appropriate antimicrobials. In 18/24 cases, the *S. aureus* isolated was clinically regarded to be a contaminant. The remaining six untreated patients died; in 5 cases, death occurred before blood culture results became available while in one case, the MRSA isolated was considered to be a contaminant. Two deaths occurred upon arrival at RCWMCH with pulmonary infections caused by MSSA noted on autopsy. The remaining four deaths were related to MRSA bacteraemia without a source; three of these were also malnourished.

Total treatment duration, which was known for 261 episodes, varied according to the clinical diagnosis. The overall treatment duration was 14 days (IQR 7-29 days). Endocarditis, pulmonary and bone or joint infections were treated for a median of 44 days (IQR 39-51 days), 13 days (IQR 7-23 days) and 40 days (IQR 32-79 days) respectively. Central venous catheter-related infections were treated for 16 days (IQR 13-28 days); 8/11 episodes required line removal due to recurrent isolation of *S. aureus*. SAB without a source was treated for a median of 9 days (IQR 6-14 days).

Outcomes

According to clinical records, 32 deaths were attributed to SAB, resulting in a case fatality rate of 8.8% over the five year period. Median time to death was 3.5 days (IQR 1.5-6.5 days). SAB without a source accounted for 59% of deaths, while pulmonary infection was responsible for an additional 25% of mortality. There were no deaths attributed to central venous catheter-related infection. MRSA accounted for 53% of deaths. Factors contributing to mortality on univariate analysis included MRSA infection (OR 3.71; CI 1.77-7.66), HIV infection (OR 2.91; CI 1.23-6.87) and malnutrition (OR 2.45; CI 1.17-5.12) (Table 5). An identifiable source of SAB infection (OR 0.3; CI 0.14-0.62), and initiating appropriate antibiotic therapy within eight hours of blood culture, had a protective effect (OR 0.32; CI 0.13-0.77). Age, polymicrobial bacteraemia and the occurrence of multiple positive cultures for *S. aureus*, were not associated with mortality. On multivariate analysis, only MRSA remained a significant risk factor for mortality (OR 3.76; CI 1.12-12.67).

DISCUSSION

This report is the first comprehensive study of the epidemiology of *S. aureus* bacteraemia in children in South Africa. International trends show increasing rates of MRSA and stable rates of MSSA bacteraemia in children over the last 20 years. In England and Wales, a large multicenter study, reported an increase in the proportion of MRSA bacteraemia from 0.9% in 1990 to 13% in 2000.[26] In the United States, Wisplinghoff et al. (2003) reported MRSA bacteraemia to have increased from 10% in

1995 to 29% in 2001 in 49 paediatric hospitals throughout the country, while Burke et al. (2009) reported a similar increase of 9% in 2001 to 24% in 2006 in California.[27,28] No published data are available for Africa on the trends of SAB in children. In this study, the incidence of SAB and MRSA bacteraemia remained fairly constant over the period 2007 to 2011. MRSA rates in children in South Africa have shown a distinct upward trend from 2% in 1974, 18% in 1992, to 26% in this study.[4,10] However, the MRSA rate reported in the present study, is lower than the 30-60% among adults and children in public sector hospitals in South Africa in 2010.[12]

Nosocomial and healthcare-associated infection accounted for approximately half of all cases of SAB. Rates of nosocomial infection increased between 2009 and 2010 possibly due to a concurrent measles epidemic in 2009-2010, and then declined in 2011. There was a marked decline in the number of healthcare-associated infections from 2008 to 2011. There was no clear reason for this decline. Changes in *S. aureus* colonization rates within the hospital may be responsible for this observation, but further investigation is required. In this study, MRSA was a predominantly nosocomial or healthcare-associated pathogen. The proportion of CA-MRSA isolates identified (6.3%), is lower than those of other South African reports.[21,22] This study, however, focused on bloodstream infections, while most cases of CA-MRSA have been reported with skin and soft tissue infections. Due to the retrospective study design, molecular typing was not available for the CA-MRSA isolates.

As reported in other studies, infants are at greatest risk of SAB, especially with regards to MRSA bacteraemia.[26,29] In our cohort, they were also more likely to present with a concomitant organism on blood culture. This susceptibility to systemic bacterial illness may be attributed to the relative immaturity of immune responses in infants. HIV-infected children also have an increased risk of bacteraemia.[30,31] A sizable proportion of our cohort (20%) was HIV-infected, of which 49% had MRSA bacteraemia. Other South African studies also demonstrated high rates of MRSA in HIV-infected children ranging from 77-100%.[5-7] This may be due to the fact that HIV-infected children are more likely to be hospitalized than other children. Several common infective markers were also analyzed but were unable to distinguish MSSA from MRSA bacteraemia (Table 1).

Bacteraemia without a source was the most common clinical diagnosis. All community-acquired bone or joint infections were MSSA in origin, which supports the empiric antibiotic choice of cloxacillin for osteomyelitis and septic arthritis at RCWMCH. Endocarditis was only diagnosed in 2.4% of episodes of SAB. Although children in this study were not screened with echocardiography, other studies have also shown that endocarditis with SAB is an uncommon event in children compared to adults.[28,29,32]

As most episodes of MRSA bacteraemia were nosocomial or healthcare-associated infections, the antimicrobial sensitivity pattern reflected predominantly multi-drug resistant MRSA isolates, which is in agreement with antimicrobial susceptibility studies on both adults and children in South Africa.[33,34] All isolates were susceptible to vancomycin, which remains the drug of choice for managing MRSA infections in

children. Notably, HIV-infected children were observed to be infected with *S. aureus* isolates that were more likely to be resistant to TMP/SMX, gentamicin and rifampicin. This may be due to the use of TMP/SMX in most HIV-infected children for prophylaxis against *Pneumocystis jiroveci* infection, while gentamicin is a common first line antibiotic agent for all children presenting to hospital with sepsis. Many children with HIV are treated with rifampicin for TB during their clinical course, which may account for the high rates of rifampicin resistance in this cohort.

The median time to appropriate therapy in this study was significantly delayed for MRSA infections, but this did not impact on mortality. Identifying children with risk factors for MRSA infection allows better guidance for empiric antibiotic therapy for nosocomial and healthcare-associated infections. In this setting, infants, malnourished children, and residents at long-term care facilities should be treated with vancomycin as part of their empiric antibiotic coverage for nosocomial infection if *S. aureus* is considered likely, until results of antimicrobial sensitivities are available. The recent clinical practice guidelines, published by the Infectious Diseases Society for America for the treatment of MRSA infections in adults and children, advise treating uncomplicated SAB with at least 14 days of appropriate antibiotic therapy.[35] The treatment duration for this category of patient was observed to be shorter in our cohort. In our study it was not possible to determine if shorter courses of treatment for SAB without a source were as effective as two weeks of therapy.

The retrospective nature of the study imposed some limitations. The number of SAB episodes reported may be an underestimate of the true incidence of disease due to non-standardized indications for blood culture, variability in blood culture volumes collected in paediatric practice, and the initiation of antibiotics in some cases prior to blood culture collection. The absence of molecular typing of MRSA isolates limited the complete evaluation of identified CA-MRSA organisms. Moreover, our study had a high incidence of concomitant organisms which were not excluded from analysis, as their presence on blood culture did not preclude the presence of a significant infection with *S. aureus*.

This study presents important clinical and epidemiological information not previously available on *S. aureus* bacteraemia in children in South Africa. Although rates of SAB and MRSA bacteraemia were stable in our population, MRSA was a significant source of nosocomial infection and was a major risk factor for mortality in children with SAB. Prospective studies that combine clinical and molecular epidemiology of *S. aureus* infections in children are warranted.

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ILLUSTRATIONS

FIGURE AND TABLE LEGENDS

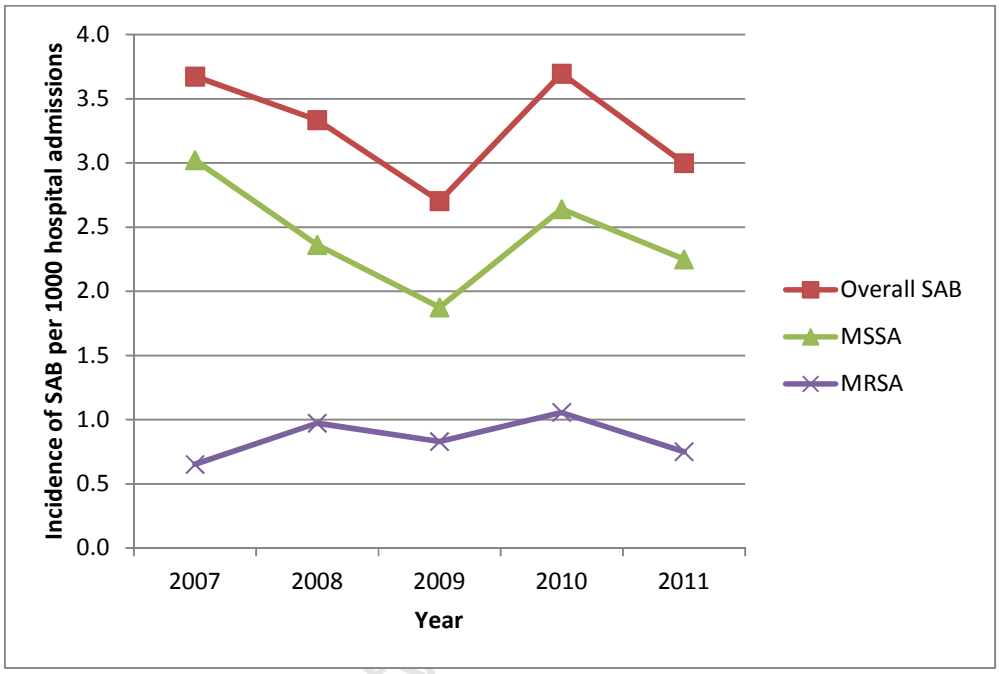

Figure 1: Incidence of *S. aureus* bacteraemia (SAB), methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) bacteraemia per 1000 hospital admissions

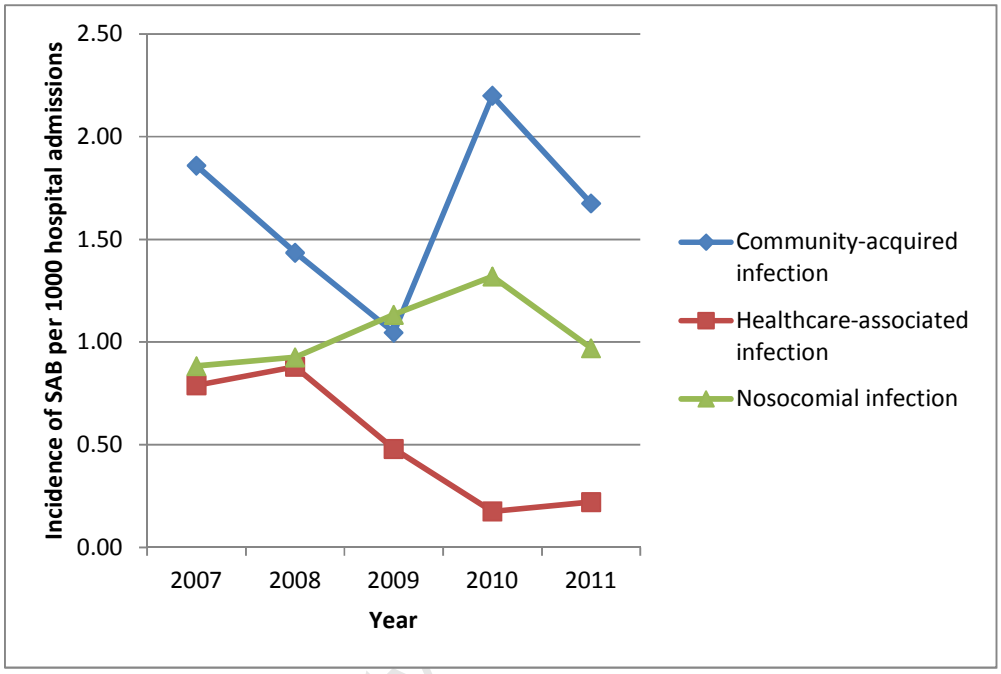

Figure 2: Incidence of community-acquired, healthcare-associated & nosocomial *S. aureus* bacteraemia per 1000 hospital admissions

Table 1: Characteristics of patients at *S. aureus* bacteraemia diagnosis

	All patients	MSSA	MRSA	p
Median age in months (IQR)	(n=365) 11.3 (3.8-42.3)	(n=270) 14.0 (3.7-51.1)	(n=95) 7.1 (3.8-19.6)	0.026
Male: Female	193:172	151:119	42:53	0.049
Median weight-for-age z-score (IQR)	(n=331) -1.53 (-3.09;-0.36)	(n=249) -1.24 (-2.35;-0.17)	(n=82) -3.00 (-4.35;-1.38)	0.0000
Underweight for age (%)	(n=331) 131 (39.6%)	(n=249) 79 (31.7%)	(n=82) 52 (63.4%)	0.0000
Median height-for-age z-score (IQR)	(n=167) -1.69 (-3.11;-0.6)	(n=103) -1.39 (-2.23;-0.5)	(n=64) -2.65 (-3.82;-1.01)	0.0002
Stunted (%)	(n=167) 76 (45.5%)	(n=103) 35 (34%)	(n=64) 41 (64.1%)	0.0000
Median weight-for-height z-score (IQR)	(n=149) -1.53 (-3.12;0.21)	(n=88) -1.40 (-3.13;0.4)	(n=61) -1.94 (-3.08;-0.01)	ns
HIV infection (%)	(n=258) 51 (19.8%)	(n=179) 26 (14.5%)	(n=79) 25 (31.6%)	0.001
HIV exposed but uninfected (%)	(n=248) 48 (19.4%)	(n=180) 39 (21.7%)	(n=68) 9 (13.2%)	ns
Median white cell count x 10^9/L (IQR)	(n=354) 14.2 (9.3-20.8)	(n=262) 14.3 (8.9-20.4)	(n=92) 13.8 (9.7-21.6)	ns

Leucocytosis (%)	(n=354) 157 (44.4%)	(n=262) 123 (47%)	(n=92) 34 (37%)	ns
Median neutrophil count x 10^9/L (IQR)	(n=277) 5.6 (2.9-10.6)	(n=204) 5.5 (2.9-10.4)	(n=73) 5.7 (2.5-11.2)	ns
Median band count x 10^9/L (IQR)	(n=268) 1.7 (0.48-3.6)	(n=198) 1.7 (0.47-3.27)	(n=70) 1.9 (0.49-4.3)	ns
Median CRP mg/L (IQR)	(n=193) 53.6 (15.2-175.5)	(n=146) 54.5 (14.4-176.7)	(n=47) 48.8 (16.1-134)	ns
Median temperature at time of blood culture ^{0}C (IQR)	(n=321) 38^0C ($37.0-38.8^0$C)	(n=246) 38.0^0C ($36.9-38.8^0$C)	(n=75) 38.1^0C ($37.3-38.9^0$C)	ns

(IQR = Interquartile range; CRP = C-reactive protein; ns= Not significant)

Table 2: Clinical diagnosis at the time of *S. aureus* bacteraemia

Clinical diagnosis at the time of culture	All	MRSA	MSSA	OR (95%CI)	p
Bloodstream infection with no source	110 (32.6%)	41 (50%)	69 (27.1%)	2.70 (1.56-4.65)	0.0001
Pneumonia & Empyema	73 (21.7%)	10 (12.2%)	63 (24.7%)	0.42 (0.18-0.89)	0.0167
Skin and soft tissue infections	58 (17.2%)	18 (22%)	40 (15.7%)	1.51 (0.76-2.91)	ns
Bone and joint infections	39 (11.6%)	2 (2.4%)	37 (14.5%)	0.15 (0.02-0.60)	0.003
Gastroenteritis	20 (5.9%)	2 (2.4%)	18 (7.1%)	0.33 (0.04-1.43)	ns
Staph scalded skin syndrome	11 (3.3%)	0	11 (4.3%)	-	-
Central venous catheter-related infection	11 (3.3%)	6 (7.3%)	5 (2%)	3.95 (0.97-16.74)	ns
Endocarditis	8 (2.4%)	2 (2.4%)	6 (2.4%)	1.04 (0.10-5.95)	ns
Meningitis	2 (0.6%)	1 (1.2%)	1 (0.4%)	3.14 (0.04-247.1)	ns
Pericardial effusion	2 (0.6%)	0	2 (0.8%)	-	-
Other	3 (0.9%)	0	3 (1.2%)	-	-
TOTAL	337 (100%)	82 (100%)	255 (100%)	-	-

Table 3: Risk factors for methicillin-resistant *S. aureus* (MRSA) bacteraemia

Risk factors for MRSA infection	Univariate Analysis		Multivariate analysis	
	Odds Ratio (95% CI)		Odds Ratio (95% CI)	
Hospitalization within 1 year	2.89	(1.79-4.68)*	0.92	(0.41-2.06)
Surgery within 1 year	1.65	(0.94-2.91)	2.28	(0.73-7.10)
Indwelling device	2.02	(1.09-3.74)*	0.87	(0.25-3.10)
Prior MRSA infection	16.8	(4.7-60.11)*	6.54	(0.91-46.98)
Resident of a care facility	12.68	(4.04-39.76)*	47.94	(7.0-326.8)*
Gender	1.6	(0.99-2.56)	1.48	(0.69-3.14)
Age (infant vs. children >1 year)	2.05	(1.26-3.34)*	2.39	(1.07-5.38)*
Duration of incident hospitalization	1.06	(1.04-1.08)*	1.06	(1.03-1.09)*
HIV-infected	2.72	(1.45-5.12)*	1.84	(0.72-4.69)
HIV-exposed but uninfected	0.55	(0.25-1.21)	-	
Malnutrition	4.45	(2.65-7.5)*	3.12	(1.40-6.96)*
Congenital cardiac disease	2.65	(1.30-5.38)*	0.37	(0.09-1.61)
Concurrent tuberculosis	6.76	(2.24-20.39)*	1.40	(0.23-8.44)
Chronic lung disease	1.53	(0.28-8.51)	-	
Chronic renal disease	0.75	(0.21-2.74)	-	
Malignancy	2.74	(0.89-8.38)	-	
Burns	1.23	(0.37-4.01)	-	
Long-term steroid/immunosuppressant use	1.54	(0.38-6.29)	-	

(*p<0.05)

Table 4: Antimicrobial susceptibility results of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) isolates

Antibiotic	Number (%) of sensitive isolates	
	MSSA	MRSA
Penicillin	15 (5.6)	0 (0)
Cloxacillin	270 (100)	0 (0)
Clindamycin	254 (94.4)	17 (17.9)
Erythromycin	251(93.3)	14 (14.7)
TMP/SMX	240 (89.2)	37 (38.9)
Vancomycin	269 (100)	95 (100)
Ciprofloxacin	263 (98.5)	44 (46.8)
Gentamicin	238 (88.8)	20 (21.3)
Rifampicin	261 (97.4)	37 (39.4)
Fusidic acid	266 (100)	91 (95.8)
Moxifloxacin	28 (100)	15 (78.9)
Linezolid	128 (100)	50 (100)
Teicoplanin	36 (100)	19 (100)
Tigecycline	28 (100)	19 (100)

Table 5: Risk factors for mortality with *S. aureus* bacteraemia

Risk factors for Death	Univariate Analysis		Multivariate analysis	
	Odds Ratio (95% CI)		Odds Ratio (95% CI)	
MRSA bacteraemia	3.71	(1.77-7.76)*	3.76	(1.12-12.67)*
HIV infection	2.91	(1.23-6.87)*	3.12	(0.99-9.78)
HIV exposed but uninfected	0.31	(0.07-1.40)	-	
Malnutrition	2.45	(1.17-5.12)*	1.7	(0.49-5.81)
Age	0.84	(0.40-1.74)		
Gender	1.98	(0.94-4.19)	1.6	(0.52-4.92)
Appropriate antibiotics within 8 hours of blood culture	0.32	(0.13-0.77)*	0.54	(0.17-1.67)
Identified source of infection	0.3	(0.14-0.62)*	0.39	(0.12-1.22)
Multiple positive cultures for *S. aureus*	1.22	(0.59-2.55)	-	
Polymicrobial bacteraemia	2.28	(0.81-6.44)	-	

*($p<0.05$)

PART D: APPENDICES

ACKNOWLEDGEMENTS

I would like to thank Professor Brian Eley and Dr James Nuttall for their invaluable guidance and supervision of the thesis from the conception of the project to the development of the final manuscript; Dr Andrew Whitelaw for providing access to the NHLS database as well as editing and contributing to the final manuscript; Sister Patricia Apolles for her unfailing support and assistance with data collection; Dr Rudzani Muloiwa for his patient advice on statistical analysis; and to the staff and children at Red Cross War Memorial Children's Hospital for allowing me the opportunity to conduct this research. I am also deeply grateful to my family for their enduring encouragement, support and guidance during the completion of this dissertation and throughout my career.

During the period of the research study, I was an infectious diseases fellow supported by PEPFAR/USAID through the ANOVA Health Institute. The study was funded by an Institute of Child Health Research Award from the School of Child and Adolescent Health, University of Cape Town.

DATA CAPTURE SHEET: EPIDEMIOLOGY OF *S. AUREUS*

Study number		Folder number	
Date of birth		Gender	Male / Female
Date of admission		Date of discharge	
Weight (kg)		Height (cm)	
Ward where Cx drawn		Date of positive *S. aureus* Cx	
Positive *S. aureus* culture	MSSA / MRSA	Time Cx was drawn	
Culture specimen			
Dates of subsequent positive *S. aureus* cultures			
Positive *S. aureus* cultures from other sites			
Concomitant organisms on blood culture			
Primary Clinical Diagnosis at time of culture			
Bacteraemia no source		Osteomyelitis	
Pneumonia		Septic arthritis	
Empyema		Gastroenteritis	
Endocarditis		Skin & soft tissue	
Meningitis		Other (specify)	
Additional clinical diagnoses			
1)		3)	
2)		4)	
Infective markers at time positive culture drawn (within 24 hours)			
WCC		Neutrophil count	
CRP		Band count	
ESR		Procalcitonin	
Temp at time of Cx		Date of peak temp	
Fever (>38˚C) duration from initiation of appropriate antibiotics	< 12 hours	24-48 hours	72-96 hours
	12-24 hours	48-72 hours	>96 hrs :
Risk factors:			
Hospitalization within 1 year of positive culture	Date:	Date:	Date:
	Dx:	Dx:	Dx:
Surgery within 1 year	Date:	Surgical Procedure	

Indwelling device		H/O MRSA infection	
Resident of care facility		Other	
HIV infection	Y / N / Not tested	HIV exposed	Y / N / Not tested
Stage of HIV infection	1 / 2 / 3 / 4	Most recent CD4 count (%)	
Treatment with ART	Yes / No	Date of CD4 count	
Date ART initiated		Defaulted ART	Yes / No
Bactrim prophylaxis	Yes / No	Date of Default	
1˚ immune deficiency		Chronic lung disease	
Malnutrition		Cystic fibrosis	
Renal disease		Malignancy	
Diabetes		Immunosuppression	
Cong. cardiac disease		Burns	
Other		Concurrent tuberculosis	
Antibiotics at the time that culture was drawn			
Antibiotic		Antibiotic	
Route of admin		Route of admin	
Date started		Date started	
Date stopped		Date stopped	
Empiric antibiotics prescribed after blood culture drawn:			
Antibiotic		Antibiotic	
Route of admin		Route of admin	
Date & time started		Date & time started	
Date stopped		Date stopped	
Antibiotic		Antibiotic	
Route of admin		Route of admin	
Date & time started		Date & time started	
Date stopped		Date stopped	
Subsequent antibiotics prescribed:			
Antibiotic		Antibiotic	
Route of admin		Route of admin	
Date started		Date started	
Date stopped		Date stopped	
Antibiotic		Antibiotic	
Route of admin		Route of admin	
Date started		Date started	
Date stopped		Date stopped	

Antibiotic Sensitivities:	Date of Cx:	Date of Cx:	Date of Cx:
Penicillin	S / I / R	S / I / R	S / I / R
Cloxacillin	S / I / R	S / I / R	S / I / R
Clindamycin	S / I / R	S / I / R	S / I / R
Erythromycin	S / I / R	S / I / R	S / I / R
Vancomycin	S / I / R	S / I / R	S / I / R
TMP/SMX	S / I / R	S / I / R	S / I / R
Ciprofloxacin	S / I / R	S / I / R	S / I / R
Moxifloxacin	S / I / R	S / I / R	S / I / R
Gentamicin	S / I / R	S / I / R	S / I / R
Rifampicin	S / I / R	S / I / R	S / I / R
Fusidic Acid	S / I / R	S / I / R	S / I / R
Linezolid	S / I / R	S / I / R	S / I / R
Teicoplanin	S / I / R	S / I / R	S / I / R
Tigecycline	S / I / R	S / I / R	S / I / R
Vancomycin MIC			
Vancomycin level/s (dates)			
Complications of infection:			
Surgical procedure + date			
ICU admission		High care admission	
Septic shock	Fluid boluses / Inotropes	Renal impairment	↑Urea / ↑Creat / Dialysis
Respiratory failure	CPAP / IPPV	Hepatic dysfunction	↑ Bili / ↑ ALT or AST / ↑INR
Other			
Death (date & cause)			
SUSPECTED CONTAMINANT	Yes / No	Transfer to another hospital	

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Observatory 7925
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e-mail: lamees.emjedi@uct.ac.za

12 January 2011

HREC REF: 029/2011

Dr R Naidoo
Paediatric Infectious Diseases
Red Cross Hospital

Dear Dr Naidoo

PROJECT TITLE: EPIDEMIOLOGY OF INVASIVE STAPHYLOCOCCUS AUREUS INFECTIONS IN PAEDIATRIC PATIENTS AT RED CROSS CHILDREN'S HOSPITAL, CAPE TOWN, SOUTH AFRICA

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Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

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Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
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Federal Wide Assurance Number: FWA00001637.
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lemjedi

Annual Progress Report

Date	16 January 2012
HREC REF Number	029/2011
Protocol number (if applicable) & Protocol title	Epidemiology of invasive *staphylococcus aureus* infections in paediatric patients at Red Cross Children's Hospital, Cape Town, South Africa
Principal Investigator	Reené Naidoo
Department / Office Internal Mail Address	Paediatric Infectious Diseases Unit, 5th Floor ICH building, Red Cross Children's Hospital

List of documentation

The study is a retrospective folder review of invasive staphylococcal infections that occurred at Red Cross Children's Hospital for a period of 5 years. My original study duration was from January 2005 until December 2009. I wish to amend the study period to January 2007 until December 2011. This would enable my research to follow more current trends of infection. I have thus far collected all data from January 2007 until December 2009. The project is expected to be completed within the next 6 months.

The original protocol is attached with tracked changes of the proposed amendment of the study time frame.

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1. Trujillo M, Correa N, Olsen K, et al. Cefprozil concentrations in middle ear fluid. *Pediatr Infect Dis J*. 2000;19:268 –270.

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5. Friedman SA. Preeclampsia. Obstet Gynecol. [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

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6. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS web site]. June 1, 1996. Available at: http://www.ama-assn.org/ special/hiv/ethics. Accessed June 26, 1997.

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